MECHANISM OF BACTERIOPHAGE INACTIVATION BY OXIDIZED SPERMINE

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Received March 10, 1965

Spermine, a naturally occurring polyamine, is oxidized by beef plasma amine oxidase (Tabor, Tabor and Bachrach, 1964) as follows:

$$\underset{\text{Spermine}}{NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2} + 2O_2 + 2H_2O \longrightarrow 2NH_3 + 2H_2O_2 +$$

(Reaction 1)

$$O{=}\underset{H}{C}{-}(CH_2)_2NH(CH_2)_4NH(CH_2)_2\underset{H}{C}{=}O$$

The oxidation product, a dialdehyde, inactivates coliphages of the T-uneven series (Bachrach, Tabor and Tabor, 1963) and M-S2, a RNA phage.

Bacteriophages may be inactivated by (a) interference with their adsorption to the host bacteria, e.g. by $Cd(CN)_2$ (Kozloff and Henderson, 1955); (b) interference with the injection of phage DNA into the host bacteria e.g. by formaldehyde (Hershey and Chase, 1952) and by protamine (Siefert-Gretchman, 1961); (c) interference with the maturation of the phage in the infected bacteria e.g. by proflavine (De Mars, Luria, Fisher and Levinthal, 1953) and by actinomycin (Nakata, 1962).

The present communication deals with the mechanism of inactivation of coliphage T_5 by oxidized spermine. It will be shown that the treated phages adsorb to the bacterial cells and inject their DNA. This injected DNA, however, does not lead to the formation of mature phages.

Oxidized spermine was prepared by oxidizing spermine with plasma amine oxidase (Bachrach, Tabor and Tabor, 1963) as follows:

Spermine, 10 µmoles, was incubated at 30° in a Warburg flask with 100 µmoles of potassium phosphate buffer pH 7.0, 0.3 ml. (300 spectrophotometric units) of a 150-fold purified amine oxidase preparation, and 150 units of crystalline catalase (Boehringer & Soehne, Mannheim, Germany) in a final volume of 3.0 ml. The reaction went to completion in about 120 min. with the consumption of the theoretical amount of oxygen (Reaction 1 above).

P^{32} labeled T_5 phages were prepared according to Hershey and Chase (1952) as follows:

Escherichia coli B was grown in 200 ml. of glycerol lactate medium (Hershey and Chase, 1952) at 37° for 4 hrs. The culture was then infected with T_5 phages in a multiplicity of infection of approximately 1.0. Three min. after the addition of phages, 50-100 μc. of P^{32} orthophosphate (The Radiochemical Centre, Amersham, England) were added. The culture was incubated with aeration for another 5 hrs., a few drops of chloroform were added, and the culture was then kept overnight at 4°. The radioactive phages were harvested and purified by 3 cycles of low and high speed centrifugations. The precipitate containing $1-5x10^{12}$ phages, was finally suspended in 7.0 ml. of adsorption medium (Hershey and Chase, 1952). For adsorption, E. coli B was grown in 250 ml. of nutrient broth for 4 hrs. The cells were harvested by centrifugation and suspended in 10-15 ml. of adsorption medium. To this suspension, 1.0 ml. of P^{32} labeled phages was added, (multiplicity of infection, approximately 1.0). After shaking at 37° for 5 min., 50γ/ml. of chloramphenicol were added and the free phages were removed by centrifugation at 3,000xg for 20 min. The precipitate, containing bacteria and adsorbed phages, was suspended in 20 ml. of blendor fluid (Hershey and Chase, 1952). The Waring Blendor experiment was carried out as described by Hershey and Chase (1952), except that a M.S.E. Homogenizer was employed for 3 min. at 16,000 r.p.m. Phages were inactivated by incubating 0.5 ml. of oxidized spermine (1.5 μmoles) with 3 ml. of phage suspension for 90 min. at 37°. Excess of oxidized spermine was removed by dialysis.

Table 1 shows that T_5 phages adsorbed to E. coli B cells and injected into the host cells 66% of their P^{32}. This value is in agreement with that obtained by Hershey and Chase (1952) who found, with T_2 phages, that 65% of phage P^{32} is injected into the host cells under similar experimental conditions. T_5 phages inactivated by oxidized spermine adsorbed to their host cells and injected 58% of their P^{32} into the bacteria (Table 1), despite a decrease of 6 logs in their titer.

In a similar experiment, H^3 spermine (Schwarz BioResearch Co., N.Y.) was oxidized by amine oxidase as above. T_5 phages were incubated with tritiated oxidized spermine (1.10^4 cpm/μmole). This labeled compound was firmly attached to the phages and did not dissociate even after prolonged dialysis. T_5 phages, thus labeled, were shown to bind $3x10^7$ molecules of oxidized spermine per phage. About 80% of the tritiated oxidized spermine, attached to the phage, was found to be bound to phage DNA. These labeled phages were incubated with E. coli B cells, which were then subjected to a Waring Blendor experiment as above. Tritiated oxidized spermine was injected into the infected cell along with

Table 1

P^{32}-Labeled T_5 Coliphages - Blendor Experiment

	Cells + phages (cpm)	Free phages (cpm)	Adsorption (%)	Blendor fluid (cpm)	DNA injected (cpm)	Ghost (cpm)	DNA injected (%)
T_5	143,685	91,460	41	72,000	53,210	26,600	66
T_5 + Oxidized Spermine	103,730	3,400	97	76,320	44,444	32,000	58

phage DNA. Subsequent extraction of DNA from infected bacteria by the duponol method (Marmur, 1963) showed that the tritiated oxidized spermine was indeed bound to the intracellular DNA.

All these experiments point to the possibility that phage DNA is

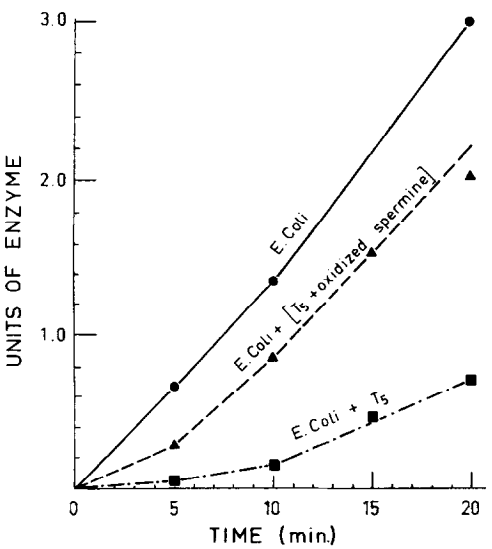

Fig. 1. Effect of inactivated T_5 phages on β-galactosidase inductions. E. coli B was grown and induced as described in the text. T_5 phages were treated with oxidized spermine and dialyzed to remove the unbound reagent. Enzyme activity is expressed in units (1 unit of β-galactosidase splits 1 mμmole of ONPG per min. at 37°) per 1.0 ml. of original culture (1.5×10^8 cells).

"inactivated" by oxidized spermine. Therefore, DNA-oxidized spermine complexes, injected into E. coli cells, should not affect the metabolism of the latter. Monod and Wollman (1947) showed that phage-infected E. coli W cells, can no longer be induced to form β-galactosidase, whereas normal uninfected cells could form β-galactosidase after induction with TMG (methyl-β-D-thiogalactoside).

E. coli B was grown in Davis succinate medium to 1.5×10^8 cells/ml. To one ml. aliquots of this suspension were added 2.0 ml. of Davis medium, 0.5 ml. of T_5 phages or 0.5 ml. of oxidized spermine treated T_5 phages, (multiplicity of infection 10-15). After incubation at 37^0 for 5 min., 0.4 ml. of 0.01 M TMG was added. Samples were tested for β-galactosidase activity (Wallenfels, 1962) at various time intervals. Induction was arrested by the addition of 200 γ chloramphenicol. Fig. 1 shows that infection of E. coli B with T_5 phages leads to an inhibition of β-galactosidase induction by approximately 75%. T_5 phages treated with oxidized spermine had only slight effect on the induction.

Our results thus indicate that oxidized spermine does neither interfere with the adsorption of T_5 phages, nor with the injection of their DNA. The injected DNA-oxidized spermine complex is, however, biologically inactive and does not markedly affect the normal metabolism of the host.

Aided by grant CA-06867 from the National Cancer Institute, United States Public Health Service.

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